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Investigating the effect of photodynamic therapy on nerves using tissue engineered culture models

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INTRODUCTION: Photodynamic therapy (PDT) shows potential as an effective treatment for prostate cancer [1]. Clinical observations indicate that this approach causes fewer nerve damage related side effects than conventional treatments. The aim here is to investigate the effect of PDT on nerve tissue using engineered 3-dimensional cell culture models. Initial experiments focussed on establishing photosensitiser localisation in neurones and Schwann cells, then developing a model for simulating nerve PDT in culture.

METHODS: Neurones and Schwann cells were cultured from the dorsal root ganglia and sciatic nerves of 200g rats. Tissues were dissociated using collagenase then seeded onto glass coverslips for

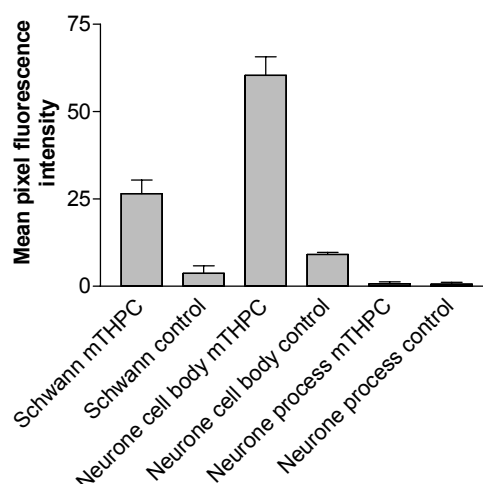
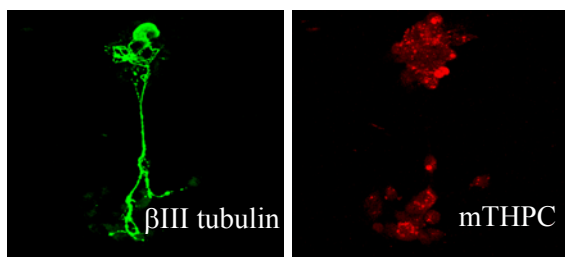


Fig 2: Quantification of mTHPC localisation in neurones and Schwann cells

DRG explants embedded within aligned Schwann cell populated collagen gels extended linear neuronal processes (fig 3), forming an effective model with which to investigate PDT in distinct parts of neurones.



localisation experiments using 4 µg/ml meso tetra hydroxyl phenyl chlorine (mTHPC) for 4 hours. Intracellular localisation of mTHPC fluorescence was visualised using fluorescence and confocal microscopy and quantified using digital image analysis. Schwann cells and neurones were identified using immunoreactivity for S100 and βIII tubulin respectively. A 3-dimensional (3D) cell culture system was developed to enable PDT to be directed against distinct parts of neurones. DRG explants were embedded within tethered aligned Schwann cell-populated type I collagen gels [2].

RESULTS: Localisation experiments revealed photosensitiser fluorescence within Schwann cells and the cell bodies of neurones, but not in neuronal cell processes (fig 1 & 2).

Fig. 1: Neurone in culture (left) with mTHPC localisation in cell body and underlying Schwann cells but not in neuronal cell process (right).

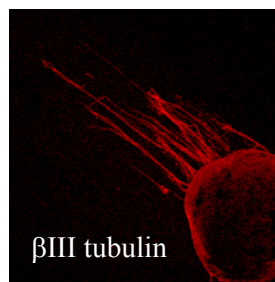


Fig 3: DRG explant extending processes in 3D culture model

DISCUSSION & CONCLUSIONS: This ongoing investigation into nerve sparing indicates that mTHPC may not be localised within neuronal processes. A 3D model has been created to investigate whether this protects the neuron from PDT damage when light is directed at the axon.

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